APPARATUS FOR AUTOMATED SYNTHESIS

1. TECHNICAL FIELD

This invention relates automated synthesizers useful in combinatorial chemistry, peptide and oligonucleotide synthesis.

2. BACKGROUND INFORMATION

Automated synthesizers capable of performing continuous reactions are particularly useful in combinatorial chemistry. These synthesizers include a robot liquid handling device for delivering chemical reactants to wells in a reaction block for stepwise syntheses.

US patent 5,746,982 discloses an apparatus for automated synthesis wherein one or more robot arms mounted on a track dispenses reagents at *x,y* axes located on a reaction block containing a plurality of reaction wells. The reaction block described therein is mounted on a stationary table optionally having an orbital mixer. US patent 6,264,891 described an apparatus for concurrent synthesis wherein reaction blocks are mounted in fixed positions on a turntable in a concentric circle equidistant from the rotatable axis. The turntable rotates in an incremental stepwise fashion to docking stations which perform physical steps in sequence. Among the limitations to the aforementioned devices is that the incremental physical steps performed on the reaction wells is one dimensional for a series of rows along an *x,y* axis containing wells. Lacking in the art is an apparatus which can perform synthetic physical steps for both rows and columns, for one or more reaction blocks each mounted on a turntable.

BRIEF SUMMARY OF THE INVENTION

It is therefore an object of the invention to provide an apparatus for automated chemical synthesis having a plurality of reaction blocks each situated on a turntable and further optionally having an arm containing an array of extending units capable of actuating

physical steps for both rows and columns. Rather than one continuous sequential reaction, the apparatus according to the invention enables one or more separate reactions to continuously proceed in parallel by possessing a plurality of actuated axes by virtue of rotational movement of one or more reaction blocks and positioning of a slidable arm containing an array of extending units capable of actuating physical steps. The apparatus is suitable for combinatorial chemical synthesis for a variety of solid phase and solution phase syntheses of small chemical molecular entities and macromolecules such as peptides and nucleic acids. The apparatus includes a platform (also referred to hereafter as a 'shaker deck') which contains a plurality of turntables, the turntables each contain a mounted reaction block. Each turntable can rotate its reaction block around a center axis 90 degrees. The reaction blocks are those known in the art and comprise a plurality of reaction wells each capable of: accepting reagent delivery into the well, drainage, and utilizing sensor devices to detect physical and chemical data as the reaction progresses. Located above each shaker deck is an arm possessing one or more extending units, such as dispensing syringes, for actuating a physical step as further described below. The arm is located above all shaker decks (the entire platform is its horizontal space) and can be positioned above each shaker deck and reaction block. The arm is capable of movement along three axes, an x,y along the horizontal plane, and a perpendicular z axis. Each incremental step situates a particular reaction well within a reaction block by movement in the horizontal axes effected by lateral movement of the apparatus arm, and/or actuated by rotation of the turntable. The vertical z axis provides for height adjustment movement of the arm for physical steps such as fluid dispensing, separation, vacuum, extraction and aspiration. The three actuated axes as described herein can be, and are preferably, controlled by a programmable computer.

The reaction steps generally include providing a plurality of reaction wells containing reagents for liquid phase chemistry or solid phase. Solid phase included for example, the core of the molecule to be derivatized attached to a solid support, or where one or more of the reagents, catalysts or purification scavengers is attached to a support. The reaction wells are contained in a reaction block, the well is translated in the x or y horizontal plane to be situated for a physical step, for example to receive fluid delivery, by rotational movement of the turntable and the translation of extending unit. Reaction is facilitated in each well by sequential reagent delivery, with subsequent independent

steps for dissolution, separation, extraction, resin delivery, resin washing, resin cleavage, filtration, a means for agitation, temperature and pressure controls and detection. Accordingly, the apparatus as described herein possesses one or more extending units, each containing a fluid delivery system and fluid drainage system, said systems being capable of solvent, resin and reagent transfers or dissolution, and possessing means for extraction and washing. The apparatus also accordingly contains turntables having a universal docking system for a diversity of reaction blocks, said turntables being capable of rotation and orbital movement. The apparatus further accordingly provides reaction blocks containing a fluid delivery system, fluid separation system and fluid drainage system. Each system also possess temperature and pressure controlling means, and a means of liquid sensing for pipette based separation of liquid phases of different densities. Facilitation of the reaction can be further achieved by an orbital shaker device.

In solid phase combinatorial chemistry, chemical libraries are created by covalent attachment of a diversity of reactants to a parent molecule attached to a solid resin support. Subsequent reagents are added and may be washed away upon completion. The desired product molecule is subsequently cleaved under suitable conditions followed by washing of the resin and isolation. During the automated synthesis, the apparatus may provide temperature, pressure controls, and agitation at the reaction block.

Each physical step in the automated synthesis provided by the apparatus according to the invention can be performed by orienting one or more reaction wells within the reaction block with the arm situated along the appropriate coordinates by actuation of one or more of the x, y and z axes. Each arm contains one or more extending units with means capable of vertical movement, and means for:
reagent delivery, separation, liquid drainage, liquid/liquid extraction, resin washing/workup, resin dispensing, resin cleavage, solvent transfers, reagent transfers, online reagent dissolution, temperature and pressure controlling, and liquid sensing for pipette based separation of liquid phases of different densities.

DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the apparatus of the invention.

FIG. 2 depicts an arm mount.

FIG 3. depicts a shaker deck system.

FIGS. 4 and **5** depict the interior view of the shaker deck system.

DETAILED DESCRIPTION OF THE INVENTION:

In one embodiment, the invention provides an apparatus for automated synthesis comprising a shaker deck comprising a plurality of reaction blocks containing a plurality of reaction wells, each reaction block being situated on a turntable, each turntable being further capable of rotating the reaction block along a central axis.

In another embodiment, the invention provides an apparatus for automated synthesis comprising a shaker deck comprising a plurality of reactionof reaction blocks containing a plurality of reaction wells, an arm comprising an array of extending units capable of actuating physical steps along a plurality of axes of each reaction block, each reaction block being situated on a turntable, each turntable being further capable of rotating the reaction block along a central axis.

In another embodiment, the invention provides an apparatus for automated synthesis comprising a shaker deck comprising a plurality of reaction blocks containing a plurality of reaction wells, an arm comprising an array of extending units capable of actuating physical steps along a plurality of axes of the reaction block said arm being situated on a slidable mount having bi-directional and lateral movement, the reaction block being situated on a turntable, each turntable being further capable of rotating the reaction block up to 90 degrees along a central axis.

In each of the above mentioned embodiments of the apparatus according to the invention, there are preferably four reaction blocks in a shaker deck, each reaction block mounted on a turntable having a universal mount for each type of reaction block, the

turntable is capable of rotating its reaction block up to 90 degrees around a center axis, said shaker deck further comprising shaker orbital agitation means and means for performing one or more steps selected from drainage, temperature control, pressure control, detection means for physical and chemical properties of products and reactants .

Other non-limiting examples of embodiments within the scope of the invention are provided herein-below.

All technical and scientific terms used in this application shall be understood to have their meaning commonly understood by those of ordinary skill in the art.

The 'physical steps' according to the invention include: reagent delivery, separation, liquid drainage, liquid/liquid extraction, resin washing/workup, resin dispensing, resin cleavage, solvent transfers, reagent transfers, online reagent dissolution, temperature and pressure controlling, and liquid sensing for pipette based separation of liquid phases of different densities. Means for implementing such physical steps are those known in the art, such as those described by Cargill et. al, Automated Combinatorial Chemistry on Solid Phase. LRA (8) 139-148 (1996); US patent nos. 4,746,490; 4,981,801; 5,147,608; 5,296,911; 5,503,805; 5,526,835; 5,609,826; 5,746,982; 5,314,825; 5,876,668; 6,264,891 and 6,420,123.

Automated synthesis according to the invention shall include combinatorial chemistry, peptide and oligonucleotide synthesis, parallel organic synthesis using both solid phase and solution phase reagents and reactions.

All citations made in this application are each incorporated herein by reference in their entirety.

II. Apparatus

A. Operating Parts

As described herein above, the arm unit is capable of actuating a plurality of physical steps of which a non-limiting example is reagent delivery.

Reagent delivery is achieved by a fluid delivery system that delivers fluid to the wells of the reaction block docked on each turntable. Referring to FIGS. **1** and **2**, the fluid delivery system includes, for example, arm unit **100** positioned above turntable **110**. The assembly arm comprising a plurality of extending units comprising modules **120** such as syringes capable of performing physical steps. In one embodiment, the module **120** is a dispensing module comprising a dispensing head **200** adapted to deliver an amount of fluid to a well in the reaction block **130** docked to the turntable. The system includes one or more tube ends such as fluid lines **140, 230** each fluid line connecting a fluid dispenser to the dispensing head. A displacement pump providing positive pressure can deliver liquid to the dispenser head.

Arm mount **150**, **210** also effectuates 2 dimensional movement in the planar *x,y* coordinates of arm unit **100** by slidable lateral movement means located in arm unit slot **220**. The arm mount can also be translated in a direction perpendicular to the slot by means capable of two way movement of the entire arm mount across the apparatus on frontal and rear support brackets **160** possessing slidable means which may be mechanized, and computer controlled. The dispenser head motion, by movement of arm **100** and turntable **110**, in relation to the reaction wells can be radial, from outer diameter to inner diameter, linear movement to the next well in the rotation, or diagonal, a combination of radial and linear. The dispenser head hovers above the center of each well designated by the synthesis program. If the program instructs the dispenser head that fluid is to be delivered to a particular well, arm **100** lowers along the vertical z axis to deliver the fluid by syringe.

The module **120** may comprise a plurality of tube ends **230** for additional physical steps to be performed. All tube ends feed into modules **120** pointing downward towards the well. For example, a plurality of fluid heads may deliver reagents to a single well sequentially at a single step, or a plurality of wells at a single step.

Liquid drainage is achieved by a drain system that removes liquid from the wells by any means. In one embodiment, the apparatus is adapted to drain liquid by top emptying or

bottom emptying. In one embodiment, the drainage is achieved by bottom emptying wherein the lower portion of the reaction block defines a manifold provided with a plurality of waste conduits as described in US patent no. 5,746,982, incorporated herein by reference. Means for drainage include any means for introducing a difference in pressure between the atmosphere above the reaction liquid and the atmosphere below the reaction liquid such as: positive pressure being introduced by the tube ends **230** causing the well contents to flow down the pressure gradient, a vacuum pump may be connected to the manifold introducing a positive difference in pressure causing the well contents to flow down the pressure gradient and thereby being flushed from the well.

Means for liquid/liquid extraction, resin washing/workup, resin dispensing, resin cleavage, solvent transfers, reagent transfers, online reagent dissolution, temperature control, pressure controlling, liquid sensing for pipette based separation of liquid phases of different densities are all those known in the art and are within the scope of the invention.

The following examples are provided by way of illustration, not limitation.

A. Introduction

Referring to FIG 3 showing a shaker deck **300**. One or more shaker decks can be installed and operated in the apparatus shown in FIG 1. On the shaker deck, a plurality of reaction blocks **310** (**130** as shown in FIG. 1) are each individually mounted on a turntable **320**, said turntables are in turn mounted within shaker deck **300**. FIGS 4 and 5 provide a further detailed description of an interior view of the shaker deck. Housing **400** supports turntables **320**, said housing is fixed along the shaft of moveable support cams **410** at one longitudinal end, which in turn are anchored to housing **420** at the other longitudinal end. FIG 5 shows another internal view of the shaker deck where housing **500** (same as **400** in FIG 4) can be shaken by means such as drive pulley **510** where said rotation is translated to a shaking motion via a cam or eccentric translation being performed evenly by means of counter balance **520**. Pulley **530** part of the shaker motor is also shown.

All chemical protocols are divided into reaction steps which, in turn, are divided into physical steps. The physical steps include delivering fluid to a well **330**, draining fluid from a well, washing a well, and incubating a well. Incubating can consist of some or all of controlling the contents of the atmosphere and the pressure of a well, controlling the temperature of a well, and agitating (mixing) a well as provided by means such as shaker motor **340**. Washing involves adding fluid to a well, agitating in most cases, and subsequently draining the well. This can be performed in sequential stations dedicated to fluid delivery, agitation, and fluid draining, or by combining all three activities at the same station. More specifically, a fluid delivery module can provide fluid delivery to a well, optionally followed by agitation means, and a vacuum line connects to a draining module, all attached by various means to the well. Temperature control, atmosphere control, and pressure control are also connected to the well via various methods. For example, a reaction step could involve adding liquid comprising reactant to a well, incubating the well for a specified period of time while agitating at a specific temperature and pressure, draining the liquid from the well, and washing the well at least once.

Once all the reaction steps in the protocol are divided into physical steps, the number of steps is adjusted to the number of wells in the apparatus which depends on the specific reaction well modules which are mounted to the apparatus, e.g, 8 reaction array modules consisting of 48 wells each for a total of 384 total wells. For example, a wash step may actually consist of delivering washing liquid to all 384 wells followed by agitation of each reaction array module, followed by draining of all wells. Then, the apparatus may be set up and prepared to deliver the appropriate reagents to the designated wells in the desired order as well as additional physical steps can be added as desired by the user in order to create the desired products, whether that product is a chemical library, a purified solid substrate, or separated liquid bilayers, for example.

In addition to the generation of chemical products, the apparatus can be used for additional liquid handling, agitation, and environmental control tasks as needed in the laboratory.

In one embodiment, the desired product is a library of small molecules synthesized on solid support. Presented here are the physical steps that one can perform on an

apparatus of this invention to carry out a generalized protocol for solid phase small molecule synthesis.

B. Small molecules on solid resin support

Small molecule synthesis on solid support generally consists of carrying out a series of chemical reaction steps where the desired product remains bonded to a large polymer that exists in solid state. This method allows for excess reagent and side products to be easily removed by flushing the resin with clean solvent in the form of a wash step prior to subsequent reaction steps. Following synthesis, the desired product is removed from the solid support through a variety of different methods depending on the type of solid support and the type of the chemical product produced. The general protocol can involve the following physical steps:

1. Deliver to each reaction well **330** a known amount of solid resin usually in the form of small beads (50-250 micron diameter).
2. Wash the resin in each well (each wash step consists of a. deliver liquid to each well, b. agitate, c. drain the liquid from each well, leaving behind the solid resin)
3. Dispense a liquid comprising the chemical reactant in solution form to each well
4. Incubate for a specific duration of time while agitating, controlling the temperature, and controlling the atmosphere of each well.
5. Drain the wells of liquid
6. Wash the resin in each well
7. Repeat steps 3 to 6 as often as needed until the synthesis is complete
8. Remove the product from the solid support and collect the product

C. Small molecules in solution phase reactions

Small molecule synthesis in solution phase

Small molecule synthesis in solution phase generally consists of carrying out a series of chemical reaction steps where the desired product remains dissolved in solution throughout the synthesis. In some cases, the product may be precipitated as a solid form in order to extract the product from the surrounding solution. Unlike solid phase

synthesis, there is not a solid bead that can easily be identified as containing the product at any given step, so the chemist uses a variety of methods for locating and extracting the desired product both at the end of the synthesis as well as in between individual steps of the synthesis. Though the actual procedures vary from synthesis to synthesis, the general protocol might involve something such as this:

1. Dispense a liquid comprising the chemical reactant in solution form to each well
2. Dispense a second reactant in similar form
3. Incubate (agitate, temperature control, atmosphere control)
4. Dispense a solvent that will form a bilayer (such as with oil and water) where the intermediate product will predominantly exist in one of the two layers.
5. Extract the layer containing the intermediate product
6. Repeat steps 1-5 as needed.

D. Reagent preparation

Beyond chemical synthesis procedures, the apparatus can be used for a wide variety of other tasks and physical steps required by the laboratory. One example of this is in the area of reagent preparation. Many chemical reagents are delivered in the form of bottles of small powders which prior to use must be dissolved to a specific concentration in a specific solvent. This involves weighing out the reagent on a scale and adding a specific volume of the solvent to the vessel containing the reagent, and then agitating to dissolve the reagent. The apparatus of the invention can be used for some of these steps to assist the user in these mundane tasks, and in fact, reagent preparation can even be added to a longer chemical synthesis method in order to provide fresh reagents during the process of the protocol.